A Fully-Automated Device for Processing Tissue into Single Cells using Electric Fields

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OVERVIEW

- **Purpose:** Automated tissue dissociation into single cells for downstream analysis.
- **Methods:** Liquid handling system, centrifuge, & electrodes for electrical dissociation of tissues. Cells stained & imaged to assess viability and recovery.
- **Results:** 96% single cells recovered in 1/15 of the time of manual dissociation.

INTRODUCTION

Tissue Dissociation: Tissue can be dissociated into its constituent single cells for downstream analysis such as single cell RNA sequencing (scRNAseq) for cancers.

Drawbacks with current approaches: Mechanical or enzymatic dissociation methods alter the transcriptomics of the cells. Existing instruments automate tissue dissociation, however these still require lengthy manual preparation steps, and have issues with purity, single cell content.

Electrical Tissue Dissociation: Previous work from our group identified the novel use of electric fields to dissociate tissues without altering transcriptomics [1].

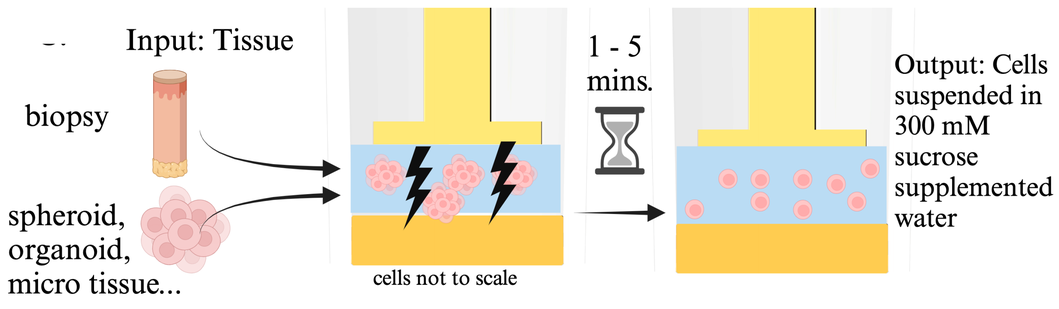

Figure 1. Electrical Tissue Dissociation into single cells from various tissue type inputs.

METHODS

Tissue: Glioblastoma Spheroids

- Human glioblastoma cells, obtained surgically at the RI Hospital, IRB #418015.
- 1 million cells cultured in a 60 mm non-treated dish, harvested after 5 days [2].
- Culture media: 1X Neurobasal A, 2 mM GlutaMAX-I, 100X Antibiotic-Antimycotic, 20 ng/mL bFGF, EGF, B-27-A, and heparin.

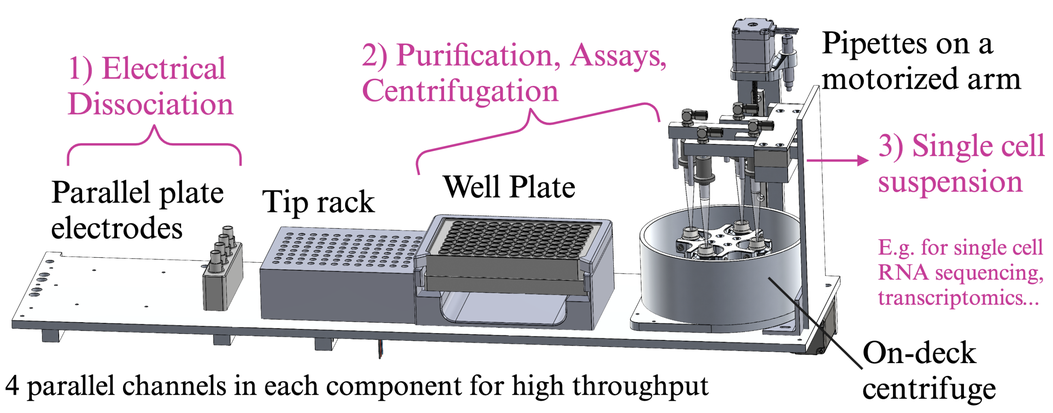

Figure 2. Automated device model depicting different components (black text) and their functions (red).

Device Design

- Robotic XZ linear motion system, 4 channel pipette assembly, on-deck centrifuge, 4x parallel electrical processing chambers with adjustable gap lengths.

Dissociation Experiments

- **Manual control:** Spheroids were spun, resuspended in StemPro Accutase, left for 15 minutes at 37°C, then physically agitated.
- **Electrical test:** Spheroids were resuspended in 300 mM sucrose supplemented water, processed at 10 V/cm, 1 kHz, AC square wave for 1 min, in device processing chambers.
- **Electrical control:** Same parameters as electrical test, but processed in a commercial electroporation cuvette (unsuited to automation).

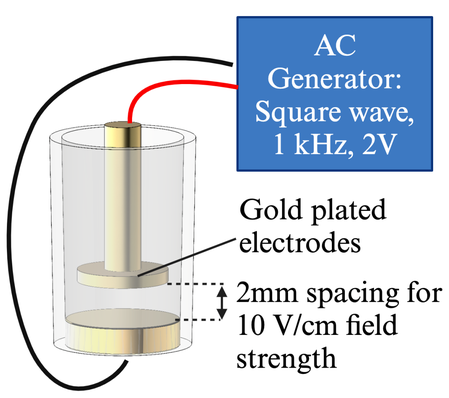

Figure 3. Electrical processing chamber's parallel plate electrodes. Polypropylene tubing, $d_{internal}$ = 6 mm (ThermoFisher). Gold plated electrodes (DigiKey). Top electrode: PC pin, d = 4.75 mm; Bottom electrode: disk, d = 6 mm.

- COMSOL multiphysics was used to model the design of the electrical processing chamber and check for uniformity in the electric field.
- Cellaca MX (Revvity) high-throughput cell counter and the ViaStain AO/PI assay was used to assess viability and cell count.
- Inverted phase contrast microscope images were taken, 5 per sample. ImageJ was used to measure aggregate size and count cells.

RESULTS

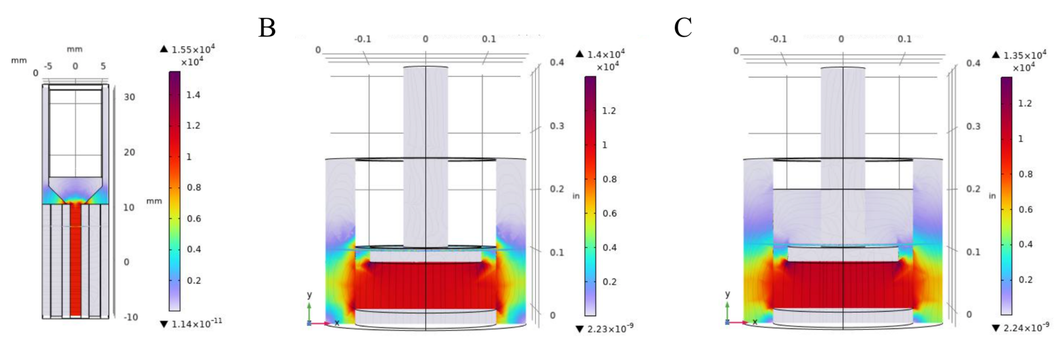

Figure 3. COMSOL analysis of the uniformity of the electric field strength (V/cm) of an electroporation cuvette (A) as an electrical control, and using our electrical processing chamber design (B) and with a liquid level that goes above the top electrode (C).

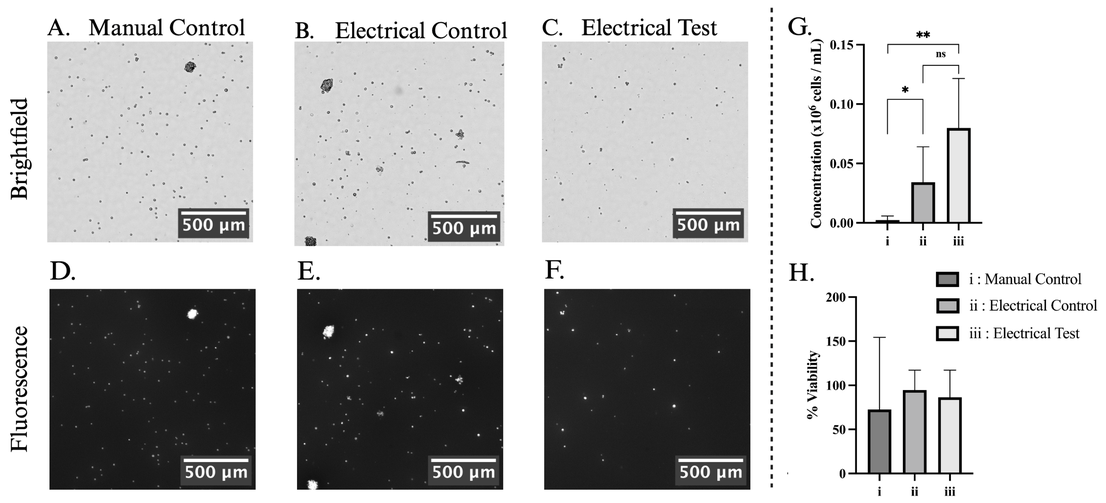

Figure 5. Cell count & viability from Cellaca MX. Brightfield images of cells dissociated manually (A), using electroporation cuvette control (B) and our reconfigured design (C). (D-F) Live/dead AO/PI stained images of the dissociated cells from the same groups. Plots comparing the number of cells dissociated (G) and their % viability (H) between processing methods.

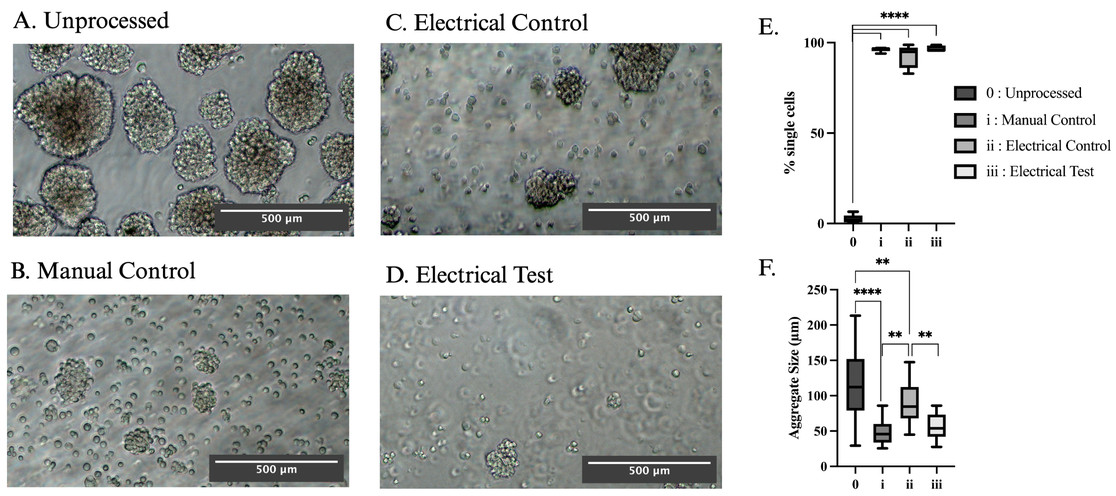

Figure 6. Dissociation analysis. A-D Brightfield images of spheroids/cells for (A) unprocessed sample, (B) manual control, (C) electrical control, and (D) the electrical test. E: % Single cells for each condition, F: Aggregate size (µm).

CONCLUSIONS

How our device's electrical processing chambers compare to manual and electrical controls:

- Obtained the highest cellular concentration recovered 0.08 ± 0.04 x 10^6 cells/mL.
- Caused no decline in viability
- **Recovered 96 ± 2% single cells** (very few aggregates)
- Aggregates were small ~53 µm compared to unprocessed spheroids ~110 µm.
- **1/15 of the time taken** for the manual workflow.

Advantages of our device's electrical processing chambers:

- Reusable
- Simple cleaning
- Autoclave compatible
- Customizable gap size
- Low cost (<$1)
- Automation friendly

Shows promise for creating a fully-automated device for dissociation of tissues into single cells.

ACKNOWLEDGEMENT

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REFERENCES

[1] E. C. Welch, H. Yu, G. Barabino, N. Tapinos, and A. Tripathi, "Electric-field facilitated rapid and efficient dissociation of tissues into viable single cells," Scientific Reports, vol. 12, no. 10728, 2022.

[2] R. R. Flores, E. C. Welch, and A. Tripathi, "Bioimpedance spectroscopy system for glioblastoma spheroid growth and dissociation characterization," in 2023 IEEE BioSensors Conference (BioSensors), 2023, pp. 1–4.